UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	**March 27, 2007**
LISZIEWICZ, et al.	:	**Atty Docket No. RGT 9771**
Serial No. 10/081,922	:	**Group 1632**
Filed: 21 Feb 2002	:	**Examiner: Wilson**

pages 32-52

For: Method of Delivering Genes into Antigen Presenting Cells of the Skin

Mail Stop Appeal Brief, Patents
Commissioner of Patents
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Response to Notification of Non-Compliant Appeal Brief [1]

In response to a Notification of Non-Compliant Appeal Brief bearing a mail date of March 13, 2007, enclosed are pages 7- 52 of the Appeal Brief. Per conversation with the Examiner, the Brief has been amended to include a separate section titled Grounds of Rejection to be Reviewed on Appeal, and to summarize the 46-page Final Rejection. The Examiner's recommendation to delete all the references to the specification by page and line number in the Summary of Claimed Subject Matter, must be declined in light of 37 CFR 41.37 (c)(1) (v) and item 5 of the Notice, as well as the assertions in the Final Rejection that this application lacks support for the Claims. Similarly, the suggestion to delete copies of evidence entered by the examiner and relied upon by appellant in the appeal must also be declined in light of 37 CFR 41.37 (c)(1)(ix), and item 8 of the Notice.

Respectfully Submitted,

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[1] This paper is being forwarded by Fax to the Commissioner of Patents P.O. Box 1450 Alexandria, VA 22313-1450 on March 27, 2007. Signed Valerie E. Looper Valerie E. Looper

polyethylenimine (Claim 26) or the manipulation of electrostatically neutral complexes to target antigen presenting cells (Claim 28) or the specific ratio of PEI to DNA that is preferred for different derivatives (Claims 30 and 42), that the glucose solution should be preferred for targeting antigen presenting cells (Claim 31), or that the range of glucose concentration in a method targeting antigen presenting cells is higher than that disclosed for general use for transfecting neurons in the Behr reference (Claims 32 and 33), or that a further step of receptor stimulation, tissue injury or cell injury might activate antigen presenting cells and therefore enhance a (disclosed in the Behr reference to be an undesired) immune response (Claim 35), or that proteins from human immunodeficiency viruses can be used in the claimed method to transfect antigen presenting cells (Claim 37), or that the claimed method would be successful using a nucleic acid sequence encoding an integration-defective or replication-defective human immunodeficiency virus (Claims 38 and 39), or that a plasmid DNA can be successfully used in the claimed method (Claim 40), or that Langerhans cells can be targeted using the claimed method and materials (Claim 41), or that use of a sugar-modified polyethylenimine would be desirable (Claim 43).

The Carson Reference (Evidence Appendix – 13)

Carson is said to provide evidence for the examiner's assertion of inherency by teaching a gene delivery complex applied to the skin that transfects dendritic cells (col. 36-37, Examples 11-12). This is not true. The definition of the complex is apparently plasmid DNA only, and is referred to repeatedly as "naked" (e.g., Col. 30, line 41). These materials were formulated in saline solution (Col. 31, line 18). Both of the cited experiments rely on injection devices: intradermal injection of plasmid (Example X, column 35, line48) or a tyne device (Col. 37, line 16). The material from the Carson reference was tested in the Behr method, and did not work. Naked DNA formulated in a glucose solution was tested for transfection into brain tissue in the Behr reference (Example 14, Col. 13, lines 9-10), and found not to work in that experiment. Similar results were obtained in an *in vitro* experiment using saline solution and cultured neurons in the Behr reference (Example 13). Also, the Carson reference reports both CTL responses and antibody responses, which it attributes possibly to the location of injection, and which indicate uptake by different classes of cells. There is no discussion whatever about how to target APCs, as opposed to other types of cells, specifically.

Furthermore, this 1994 reference can be placed in perspective by the discussion in the present specification at page 6, line 8 et seq., of Arthur, et al "A Comparison of gene transfer methods in human dendritic cells," Cancer Gene Therapy, v. 4, No. 1, 1997 pp 17-25[2], which reports that the then-known *in vitro* methods of gene transfer suffered from low efficiency. That article reported that, of a variety of gene transfer methods were tried for human DC, only adenoviral vectors were a promising vehicle for genetically engineering human DCs (Abstract). The application also discloses at page 6, line 14-19 that, as of 1998, "known *in vivo* methods include ... intradermal subcutaneous and intramuscular injection of DNA. None of these methods have been shown to effectively deliver genes into antigen presenting cells, such as dendritic cells, much less delivery of genes through the skin into the Langerhans cells."

The present Invention

The present application is a division of United States Patent No. 6,420,176, which was drawn to a novel DNA complex for gene delivery. The present application relates to a method of transfecting antigen presenting cells, the steps comprising

selecting a gene delivery complex that targets antigen presenting cells comprising DNA and one or more compounds selected from the group consisting of sugars, polyethylenimine, and polyethylenimine derivatives,

and applying the complex to the skin or mucosa surfaces of an animal,

wherein said DNA comprises a nucleic acid sequence encoding at least one immunogenic protein operatively linked to a promoter.

An advantage enjoyed by this invention is that the gene delivery complex need not be injected, but merely applied to the skin, according to the claimed method. The inventors have found, and the text of the application contains, experiments that demonstrate that both the wholly novel complex (DNA plus mannosylated PEI) and several other arguably non-novel complexes (DNA plus modified PEI, PEI with sugars, PEI alone, and sugars alone) also work in an elegant method for stimulating the immune system that does not rely on injection, added irritants or toxins, use of cultured cells or expensive new equipment such as a gene gun. The present application also discloses how to modify the teachings of the Behr reference so that the claimed

[2] This reference has been of record in the present case, as acknowledged by the Examiner on 8/9/01. A copy of the reference is enclosed at Evidence Appendix 10.

antigen presenting cells can be targeted via the mannose receptor as opposed to the asialoglycoprotein receptor (at least at page 14, line 37 – page 15, line 15, and Example 6). These cells are disclosed to be capable of producing a CTL response (page 11, line 21) and proven to do so *in vitro* (Example 3), and *in vivo* (Example 4), and additional data submitted by the inventors shows that the immune response induced in this manner does not include antibody (humoral) responses using either the (injected) *ex vivo* or (applied to the skin) *in vivo* procedures. Lisziewicz, et al., "Induction of Potent Human Immunodeficiency Virus Type 1-Specific T-Cell-Restricted Immunity by Genetically Modified Dendritic Cells"[3] J Virol, Aug 2001, p.7621-7621-7628, at Abstract and p 7626, lines 12-15, 1st full paragraph. See also Lisziewicz, et al., "DermaVir: A Novel Topical Vaccine for HIV/AIDS J Inv Dermatology, 2004,[4] p. 6, col. 1, first paragraph, last 4 lines.

C. Examiner's Arguments

The Examiner has not pointed to any disclosure or discussion within the Behr reference relating to the claimed method, nor of any disclosure that would guide one of ordinary skill in the art to choose from the many options available, to make the claimed invention or obtain its advantages. The Examiner has relied, however, on two arguments in an effort to make the rejection.

1. Inherency – Luciferase as an Immunogen

The Examiner points to Example 14 of the Behr patent, where a plasmid encoding a marker gene for luciferase was diluted in 5% glucose solution to about 3% glucose, and then diluted with PEI and injected into the brains of newborn mice, whose brains were subsequently an assayed for light emission as a sign of transfection into neurons. The reference comments that this experiment shows the advantages of the compositions of that experiment for gene therapy, that the plasmid with PEI was transferred efficiently into the brain of mice, and that no significant luciferase activity was observed when the plasmid alone was used.

There is no disclosure or discussion of antigen presenting cells, no disclosure or discussion of how to specifically target antigen presenting cells as opposed to neurons, no experiment reporting an immune response, yet the Examiner argues without citation that

[3] Evidence Appendix 6
[4] Evidence Appendix 4

Luciferase is inherently an immunogen, and that because the Behr reference taught elsewhere and without more, "topical application," that the Behr reference inherently teaches that the material in Example 14, *if placed on the skin* would target *antigen presenting cells*, because antigen presenting cells are in the skin. At best, this is an argument that it might have been obvious to try an experiment, and does not rise to the clear disclosure required to anticipate the claims.

The present application at page 6, lines 4-11 cites to a fairly contemporaneous reference (Arthur, et al.,) that indicates that different types of cell lines have different responses to transfection techniques and that of the methods screened, only adenoviral vectors showed promise for genetically engineering human DCs. (Abstract), and the application states that that the known techniques and materials had not been shown to effectively deliver genes into antigen presenting cells, such as dendritic cells, much less delivery of genes through the skin into the Langerhans cells. There is no reason to discredit this disclosure, especially in view of the Examiner's vigorous urging that the art at the time was unpredictable. Thus it is not clear that the unaltered material disclosed in Experiment 14 would inherently transfect antigen presenting cells.

It is not at all clear that luciferase will inherently cause an immune response. Luciferase is a commercially available reagent from, among many others, BD Biosciences, who characterize the material as follows on their web site[5]:

> http://www.bdbiosciences.com/pharmingen/products/display_product.php?keyID=76
>
> Luciferase Reporter Assay Applied Reagents
>
> Since the firefly beetle (Photinus pyralis) luciferase gene was introduced to molecular biology, it has provided a method of utilizing biological light production as a tool for research. Luciferase interacts with its cognate substrate luciferin to produce light emission peaking at 562 nm. For use in the laboratory, this form of luminescence can yield a very sensitive non-radioactive assay. Firefly luciferase can be reliably expressed from various expression vectors and in a diversity of organisms as a reporter in studies of gene regulation. Luciferase reporter assay systems are currently one of the best non-toxic, rapid and sensitive methods to measure gene expression. The assay is based on the detection of luciferase activity which correlates with transcription due to DNA regulatory elements in genes, mutations within those elements as well as responses to extracellular and intracellular signals. (emphasis added)

CTL responses are cellular immune responses. For the purposes of gene therapy they are considered toxic because they result in the elimination of the cells expressing the DNA, which are considered to be the "cured" or "therapeutic" cells. (At minimum, elimination of the cured cells would result in elimination of the therapeutic and prophylactic benefit). That is why the Behr reference taught that immunogenicity is to be avoided in this context (Col. 1, line 51). If marker genes were used that resulted in a CTL response *in vivo*, the transfected cells might be destroyed fairly quickly, and the experiment might appear to fail. Thus a desirable marker gene is one that is likely to provoke little, if any, immune response, to avoid interfering with the tests.

It is noted that the Applicants have used a different marker gene in their experiments, a green fluorescent protein gene derived, it is believed, from jellyfish. Such a gene was used to produce a "GFP bunny," that is, a rabbit that glows in the dark, if stimulated by the proper light source[6]. http://www.ekac.org/gfpbunny.html#gfpbunnyanchor The Examiner's statement to the contrary, if this marker gene inherently stimulated an immune response, assuming the animal could have been produced at all, the rabbit would have died from an autoimmune reaction shortly after it began to produce the protein. This existence of this genetically altered animal indicates that not all marker genes are inherently cause immune responses, and so one of ordinary skill in the art would not read the experiment to show that the material disclosed in Example 14 would inherently cause an immune response. The Examiner's assumption, that all proteins inherently cause immune (toxic) responses, has been fairly met because the applicants have pointed out that not all proteins cause such responses, and the genes that encode some of them have been found and used for marker genes.

2. *Expectation of Success*

Carson is said to provide evidence for the examiner's assertion of inherency by teaching a gene delivery complex applied to the skin that transfects dendritic cells (col. 36-37, Examples 11-12). Both of these experiments rely on devices: intradermal injection of plasmid in saline solution (Example X, column 35, line48) or a tyne device (Col. 37, line 16). The "complex" as discussed above, is "naked" DNA, which was tested in the Behr reference and found ineffective. This reference adds nothing to the Behr reference.

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[5] Evidence Appendix 8B
[6] Evidence Appendix 9

Furthermore, this 1994 reference can be placed in perspective by the discussion in the application at page 6, first full paragraph, of Arthur, et al "A Comparison of gene transfer methods in human dendritic cells Cancer Gene Therapy, v. 4, No. 1, 1997 pp 17-25, which states that that indicates that different types of cell lines have different responses to transfection techniques reports that the then-known *in vitro* methods of gene transfer suffered from low efficiency. The application also discloses at page 6, line 14-19 that, as of 1998, "known *in vivo* methods include ... intradermal subcutaneous and intramuscular injection of DNA. None of these methods have been shown to effectively deliver genes into antigen presenting cells, such as dendritic cells, much less delivery of genes through the skin into the Langerhans cells." See also Pollard, et al., "Polyethylenimine but not cationic lipids promotes transgene delivery to the nucleus of mammalian cells" J. Biol. Chem. Vol. 273, No. 13, pp. 7507-7511 (1998) discussed at page 15, lines 3-6 of the application. [7] One of the conclusions drawn by the authors of that 1998 article was that barriers to gene transfer vary with cell type (Abstract, and p. 7510, col. 2, last full para, lines 1-8 UP). This disclosure is especially credible in light of the Examiner's vigorous insistence, in another context, that the art was at the time unpredictable. The Carson reference, which related to intradermal and intramuscular injection of DNA had not been shown to be effective as of several years later. It does not yield any indicator that the Behr reference could be successfully altered in the manner disclosed in the present application, and so cannot be relied upon to support an inherency argument.

D. Analysis and Conclusion

The Behr reference does not disclose the claimed method at least because it does not indicate how to pick and choose among its teachings to transfect a different class of cells, dendritic cells, via a different receptor, the mannose receptor, directed to what is for the Behr reference, a toxic response, much less any of the details found in the dependent claims. Example 14 of the Behr reference does not include a material that one of ordinary skill in the art at the time the invention was made would interpret to inherently cause an immune response. The gene is a marker gene, and marker genes are selected for their low toxicity, one aspect of which is lack of tendency to produce immune responses. The Carson reference cannot reasonably be said to yield a reasonable expectation of success, much less the degree of certainty needed to maintain

[7] Evidence Appendix 11

an inherency rejection, to an experiment reconstructed by hindsight from the Behr reference. That is because subsequent peer-reviewed publications by others, the Behr reference itself, and the text in the present application all disclose that the techniques used in the Carson reference had not been shown to yield effective transfection in antigen presenting cells as of the file date of the present application.

6. Obviousness – Claims 23-26, 28, 30-32, 37, 40, 41 and 43

Claims 23-26, 28, 30-32, 35, 37-41 and 43 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Behr (US Patent 6,013,240, Jan. 11, 2000) as supported by Carson (US Patent 5,679,647) and in view of Holler (US Patent 5,908,923).

The Behr and Carson references are used as discussed above. The Holler reference is said to have taught a plasmid encoding a replication-defective HIV that was integrase defective for use in vivo. The combined teachings of Behr and Holler said to provide a reasonable expectation of successfully transfecting cells because Holler transfected CEM (a lymphoblastoid cell line) with integrase-defective HIV.

The phrase "transfecting antigen presenting cells" in the preamble is not being considered to bear patentable weight in considering the art because it may not occur. [sic]

Claims 25, 26 and 43 are said to be included because they are not limited to a compound that is mannosylated PEI or PEI "from a PEI 22 kDA;" claims 25, 26 and 43 encompass non-sugar-modified PEI solubilized in glucose as in parent claim 24.

Claims 28 and 30 are said to be included because Behr is said to have taught that between 5-20 equivalents of PEI amines are used relative to one DNA phosphate (col. 8, lines 15-19). The instant specification teaches that the ratio of 5:1 cause the complex to be electrostatically neutral, (¶ bridging pg 21-22).

Claims 35 and 41 are said to be included because administering the complex to the skin/mucosa as taught by Behr inherently would activate APCs by toxin activation. Cells would start expressing luciferase and this firefly "toxin" would be recognized as foreign by the animal, thereby activating APCs, including Langerhans cells.

Response – Obviousness – Claims 23-26, 28, 30-32, 37, 40, 41 and 43

A. Applicable Law

Whether patents are allowable in a given particular field of art is not a question of Patent and Trademark Office discretion but of law, and examiners have no discretion to deny patents to inventions meeting the statutory criteria. *Animal Legal Defense Fund v. Quigg*, 18 USPQ 2d 1677, 1685, Fed. Cir. (1985).

Office policy is to follow *Graham v. John Deere Co.* in the consideration and determination of obviousness under 35 U.S.C. 103. The four factual inquires enunciated therein as a background for determining obviousness are as follows:

(A)Determining the scope and contents of the prior art;

(B)Ascertaining the differences between the prior art and the claims in issue;

(C)Resolving the level of ordinary skill in the pertinent art; and

(D)Evaluating evidence of secondary considerations. (MPEP 2141)

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art to modify the reference or combine the reference teachings. Second, there must be a reasonable expectation of success. Third, the prior art combination must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the expectation of success must be both found in the prior art, not in the applicant's disclosure. MPEP 2143. "There are three possible sources for a motivation to combine references: the nature of the problem to be solved, the teachings of the prior art, and the knowledge of persons of ordinary skill in the art." *In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1457-58 (Fed. Cir. 1998) (The combination of the references taught every element of the claimed invention, however without a motivation to combine, a rejection based on a prima facie case of obvious was held improper.). The level of skill in the art cannot be relied upon to provide the suggestion to combine references. *Al-Site Corp. v. VSI Int'l Inc.*, 174 F.3d 1308, 50 USPQ2d 1161 (Fed. Cir. 1999) (MPEP 2143.01 I).

"In determining the propriety of the Patent Office case for obviousness in the first instance, it is necessary to ascertain whether or not the reference teachings would appear to be sufficient for one of ordinary skill in the relevant art having the reference before him to make the proposed substitution, combination, or other modification." *In re Linter*, 458 F.2d 1013, 1016, 173 USPQ 560, 562 (CCPA 1972). Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly

or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1071, 5 USPQQ2d 1596 (Fed. Cir. 1988).

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990) (MPEP 2143.01 III).

If the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984)
If the proposed modifications or combination of the prior art would changed the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959); see also MPEP 2143.01 V.

B. Factual Background: The Scope and Content of the Prior Art, the Differences between the Claimed Invention and the Prior Art

The Behr Reference (Evidence Exhibit – 12)

The Behr reference relates to the use of PEI as an adjuvant for gene therapy (Abstract), preferably in conjunction with plasmid DNA, although a wide variety of other materials are disclosed as well. Gene therapy is disclosed to consist in correcting a deficiency or an abnormality (mutation, aberrant expression, and the like) or in effecting the expression of a protein of therapeutic value by introducing genetic information into the affected cell or organ (Col. 1, lines 11-15). Gene therapy is a field distinct from the subject matter of the present invention, which is immunotherapy, and the reference discloses that immunogenicity is to be avoided in this context (Col. 1, line 51). Thus the entire discussion of whether the GFP marker gene used in the Behr reference could be construed as an immunogenic protein is inapt. First, no such reaction was shown. Second, such a reaction would have rendered the experiments a failure due to toxicity. If the cells inherently provoke an immune response in vivo, they get killed off very rapidly, and do not produce measurable amounts of the marker protein.

The reference states that PEI can be used in a wide variety of cells, (tumor cells, liver cells, haematopoietic cells Col. 5, lines 41-43), in a wide variety of configurations, including a wide range of amine to phosphate mol ratios (0.5 – 50 at Col. 2, line 50) without any distinction as to what might be accomplished by varying such ratios, using a wide variety of targeting elements (sugars, peptides, oliconucleotides, or lipids Col. 5, lines 55-57; sugars are listed as

useful for targeting the asialoglycoprotein receptors at Col 5, lines 64-65), for a wide variety of purposes (for example, the production of therapeutic products including enzymes, blood derivatives, hormones, lymphokines,...growth factors, neurotransmitters...synthetic enzymes, etc,, -- a list that includes thousands of items. See Col. 3, lines 29-44. Antigenic peptides are also listed at Col. 3, line 57-67, as well as antisense genes (Col. 3, line 45), sequences (Col. 4, line 1, and upstream signals to control therapeutic genes (Col. 4, lines 25-29) and that it can be used in formulations with a view to topic, cutaneous, oral, rectal, vaginal, parenteral, intranasal, intravenous, intramuscular, subcutaneous, intraocular, transdermal, and the like (Col. 6, lines 1-4) in formulations that that might be isotonic sterile solutions, dry, water or saline (Col. 6, lines 9-12 as appropriate *to enable injectable solutions to be formed* (line 13, emphasis added). Both saline (Example 13 and glucose Example 14) formulations are disclosed, without any distinction as to any advantage that might be obtained. Both direct injection and topical administration are said to be preferred (Col. 6, lines 5-9), but only direct injection into the brain for the transfection of neural tissue is shown in any experiments, and there is no disclosure of how to accomplish gene delivery by means of topical administration, or any disclosure whatever of the transfection of antigen presenting cells, or the provocation of any type of an immune response.

This reference has disclosure consistent with that for a new material or a new use for a material with potentially wide application in a given field. What is beyond the scope of this reference is specific instruction as to how to realize the full potential of the material, that is, how to obtain the results that are potentially available from it, in areas that were not of direct interest to the inventors of the reference at the time. The Examiner's statement, that the Behr reference is relied upon for the step of applying a plasmid encoding an HIV protein to the skin of an animal, is ineffective because the Behr reference shows no such thing.

Claim Limitations Missing from the Behr reference

Among the differences between this reference and the presently claimed invention are that the reference does not disclose the transfection of antigen presenting cells, or the targeting of antigen presenting cells, a most significant subset of cells, and prominent by its omission, topical application of anything, or formulations that can be used for needleless, *in vivo* delivery of genes into any cells, much less antigen presenting cells, or any *in vivo* method of delivery except injection.

Similarly, the reference does not disclose that glucose and PEI derivatives could be used in the claimed method (Claim 24) or that the PEI derivatives can target the mannose receptor instead of the asialoglycoprotein receptors (Claim 25), or anything about mannosylated polyethylenimine (Claim 26) or the manipulation of electrostatically neutral complexes to target antigen presenting cells (Claim 28) or the specific ratio of PEI to DNA that is preferred for different derivatives (Claims 30 and 42), that the glucose solution should be preferred for targeting antigen presenting cells (Claim 31), or that the range of glucose concentration in a method targeting antigen presenting cells is higher than that disclosed for general use for transfecting neurons in the Behr reference (Claims 32 and 33), or that a further step of receptor stimulation, tissue injury or cell injury might activate antigen presenting cells and therefore enhance a (disclosed in the Behr reference to be an undesired) immune response (Claim 35), or that proteins from human immunodeficiency viruses can be used in the claimed method to transfect antigen presenting cells (Claim 37), or that the claimed method would be successful using a nucleic acid sequence encoding an integration-defective or replication-defective human immunodeficiency virus (Claims 38 and 39), or that a plasmid DNA can be successfully used in the claimed method (Claim 40), or that Langerhans cells can be targeted using the claimed method and materials (Claim 41), or that use of a sugar-modified polyethylenimine would be desirable (Claim 43).

The Carson Reference (Evidence Appendix – 13)

The Carson reference is said to provide evidence for the examiner's assertion of inherency by teaching a gene delivery complex applied to the skin that transfects dendritic cells (col. 36-37, Examples 11-12). The definition of the complex is apparently plasmid DNA only, and is referred to repeatedly as "naked" e.g., Col. 30, line 41. These materials were formulated in saline solution (Col. 31, line 18). Both of the cited experiments rely on devices: intradermal injection of plasmid (Example X, column 35, line48) or a tyne device (Col. 37, line 16). Naked DNA formulated in a glucose solution was tested in the Behr reference Example 14, Col. 13, lines 9-10, injected and found not to work in that experiment. Similar results were obtained in an in vitro experiment using saline solution in the Behr reference at Example 13, Col. 12, lines 39-41). Also, the Carson reference reports both antibody responses (Col. 31, lines 54-56) and CTL responses (Example IX) which it attributes to the location of injection, intradermal rather

than intramuscular. There is no discussion whatever about topical application, or how to target APCs according to the manner of the claimed invention. Further, it is noted that the later, Behr reference discussed above, tested the materials and methods of the Carson reference and found them ineffective.

The Holler Reference (Evidence Appendix – 14)

USPN 5,908,923 to Holler, et al. discloses and claims a sequence listing for a specific transdominant negative integrase gene which is said to be capable of making at least one cell resistant to a retroviral infection. This gene was used *in vitro* to transfect a lymphoblastoid cell line. The Examiner admits that this reference does not disclose any *in vivo* method.

The methods of transfection mentioned are calcium phosphate co-precipitation, cationic liposomes, electroporation, receptor mediated endocytosis, naked DNA, transduction by viral vector, and particle-mediated gene transfer. The only method discussed, (which is also shown in the Examples) is calcium precipitation.

This disclosure simply amounts to a suggestion that the gene is useable. It says nothing about the claimed method. Indeed, this 1994 reference would appear to recommend that the gene can be successfully delivered by any and all methods. See Col. 7 lines 40-57. However, the present application discloses that an article published several years later compared transfection rates in antigen presenting cells and a cancer cell line (melanoma) that was known to be readily transfected by all the methods tested. (page 21, lines 2-4). This article reported only "low efficient" *in vitro* methods were known at the time, see page 6, lines 4-11 (cite to Arthur, J. F. et al., Cancer Gene Therapy 4:1 17-21, 1997 and Song, E. S., et al., PNAS USA 94:5, 1943-8, 1997[8]); and that neither they nor the known *in vivo* methods had been shown to effectively deliver genes to antigen presenting cells, much less delivery of genes through the skin into the Langerhans cells. See page 6, lines 16-19. Thus, this reference adds nothing to the cited combination.

The Examiner has stated that the applicants' comment that the Holler reference recommends the gene can be successfully delivered by any method, and that this is not persuasive because certain "low efficient" methods cited in the background section of the present application were said to be "successful." First, the application text discloses that these

experiments were not successful. The application discloses that they had not been shown to effectively deliver genes to antigen presenting cells, much less delivery of genes through the skin. Further, the reference does not teach the claimed method, and it is the method, not the raw materials, that is lacking in the prior art. Christening the prior art "successful" doesn't change the fact that the new method is not disclosed in the prior art. The reference does not differentiate among types of cells, methods of gene delivery, or provide any basis to choose the present method from among many, successful or not. The claimed invention is not a given retrovirus, nor is it an adjuvant. It is a method of transfecting antigen presenting cells.

The Claimed Invention

A method of transfecting antigen-presenting cells, the steps comprising selecting a gene delivery complex that targets antigen presenting cells, comprising DNA and one or more compounds selected from the group consisting of sugars, polyethylenimine, and polyethylenimine derivatives, and applying the complex to the skin or mucosa surfaces of an animal, wherein said DNA comprises a nucleic acid sequence encoding at least one immunogenic protein operatively linked to a promoter.

An advantage enjoyed by this invention is that the gene delivery complex need not be injected, but merely applied to the skin, according to the claimed method. The inventors have found, and the text of the application contains, experiments that demonstrate that both the wholly novel complex (DNA plus mannosylated PEI) and several other arguably previously disclosed complexes (DNA plus modified PEI, PEI with sugars, PEI alone, and sugars alone) also work in an elegant method for stimulating the immune system that does not rely on injection, added irritants or toxins, use of cultured cells or expensive new equipment such as a gene gun.

Differences between the Claimed Invention and the Prior Art

Among the differences between the presently claimed invention and the primary reference (Behr) are that the reference does not disclose the transfection of, or targeting of, antigen presenting cells, a most significant subset of cells, and prominent by its omission, or formulations that can be used for needleless, *in vivo* delivery of genes into any cells, much less antigen presenting cells, or any *in vivo* method of delivery except injection. The reference does

[8] Evidence Appendix 10

not teach or suggest the use of sugars to target the mannose receptor, the significance of having an electrostatically neutral complex in this context, the significance to the inventors' method of the ratio of nitrogen to phosphate, use of glucose solutions in the claimed range, any reason to prefer a glucose solution over a saline solution, or any further steps to enhance the response of the skin to the formulation. The other references do not supply any of the missing information.

The present application discloses how to modify the teachings of the Behr reference so that that the claimed method targets antigen presenting cells instead of neurons (at least at page 14, line 37 – page 15, line 15, and Example 6), in part by using a specific type of formulation based on multiple factors including the use of sugar-modified formulations and manipulation of a nitrogen/phosphate mol ratio to target a different receptor from that suggested by the Behr reference, and in part by refraining from using any injection method, that is, placing the formulation on the skin, and in part by using DNA encoding a specific class of materials undesired for the purposes of the Behr reference, immunogenic proteins. The transfected APC are capable of producing a CTL response (page 11, line 21), which is toxic for the purposes of the Behr reference (Col. 1, line 51), Additional data submitted by the inventors shows that the immune response induced in this manner does not include antibody (humoral) responses using either the (injected) *ex vivo* or (applied to the skin) *in vivo* procedures. Lisziewicz, et al., "Induction of Potent Human Immunodeficiency Virus Type 1-Specific T-Cell-Restricted Immunity by Genetically Modified Dendritic Cells"[9] J Virol, Aug 2001, p. 7621-7628, at p 7626, lines 12-15, 1st full paragraph. See also Lisziewicz, et al., "DermaVir: A Novel Topical Vaccine for HIV/AIDS J Inv Dermatology, 2004,[10] p. 6, col. 1, first paragraph, last 4 lines, and second paragraph, lines 2-4.

C. Analysis

1. The Prima Facie Case – the References and their Combination

The present rejection does not establish a *prima facie* case of obviousness against the amended claims because the claimed method is not present in any of the references individually, or their combination. As discussed in more detail in the 35 USC § 102 rejection above, the Behr reference does not inherently disclose the claimed method, with or without the support of

[9] Evidence Appendix 6
[10] Evidence Appendix 4

the Carson reference. The Behr reference must be modified in order to derive the claimed invention. The present application discloses how to modify the teachings of the Behr reference so that that the claimed method targets antigen presenting cells (a different class of cells from neurons) (at least at page 14, line 37 – page 15, line 15, and Example 6), which are capable of producing a CTL response (page 11, line 21), which is toxic for the purposes of the Behr reference (Col. 1, line 51), in part by using a specific type of formulation to target a different receptor from that suggested by the Behr reference, in part by using DNA encoding a material undesired in the Behr reference, namely an immunogenic protein, and in part by refraining from using any injection method, that is, by placing the formulation on the skin.

The question presented here, therefore, is whether the cited references contain teachings sufficient for one of ordinary skill in the art to conclude the proposed modifications would work, without rendering the Behr reference inoperative.

The Carson reference is said to teach a gene delivery complex applied to the skin that transfects dendritic cells. This is not strictly true, for the "complex" is just plasmid DNA, and both of the experiments relied upon by the Examiner use injection devices. Further, plasmid DNA alone, in both saline (Experiment 13) and glucose solution (Experiment 14) was used in the Behr reference and found to be ineffective. And the Applicants have submitted evidence that, by the time the present invention was made, it had become known that different types of cells had different sensitivities to transfection, so that the older reference would not be considered by one of ordinary skill in the art to assure success with another. See Arthur, et al "A Comparison of gene transfer methods in human dendritic cells" Cancer Gene Therapy, v. 4, No. 1, 1997 pp 17-25[11], which reports that the then-known *in vitro* methods of gene transfer suffered from low efficiency. The application also discloses at page 6, line 14-19 that, as of 1998, "known *in vivo* methods include ... intradermal subcutaneous and intramuscular injection of DNA. None of these methods have been shown to effectively deliver genes into antigen presenting cells, such as dendritic cells, much less delivery of genes through the skin into the Langerhans cells." See also the Pollard reference discussed at page 15, lines 3-6. [12] One of the conclusions drawn by the authors of that 1998 article was that barriers to gene transfer vary with cell type (Abstract, and p. 7510, col. 2, last full para, lines 1-8 UP). This disclosure is especially credible in light of the

[11] Evidence Appendix 10
[12] Evidence Appendix 11

Examiner's vigorous insistence, in another context, that the art was at the time unpredictable. Thus the Carson reference is only useful as a disclosure of a raw material that has not yet been made to work.

The Examiner does not assert that the Holler reference teaches anything about the claimed method, only that it teaches the existence of a plasmid encoding a replication-defective, integrase defective HIV. Assuming the raw material is as described, there is still no description of the claimed method.

Neither the secondary nor tertiary reference, nor their combination, present any modification to the base reference, other than to suggest raw materials. They have nothing to add to the claimed method. The *prima facie* case for obviousness has not been made, and this rejection must be withdrawn.

2. Secondary Considerations – Objective Evidence of Non-Obviousness

Objective evidence or secondary considerations such as unexpected results, commercial success, long-felt need, failure of others, copying by others, licensing, and skepticism of experts are relevant to the issue of obviousness and must be considered in every case in which they are present.

A vaccine according to the currently claimed invention is in human clinical trials in two countries. It is noted that clinical trial results are expressly NOT required according to the MPEP, however, the MPEP also acknowledges that "Before a drug can enter human clinical trials, the sponsor, often the applicant, must provide a convincing rationale to those especially skilled in the art (e.g., the Food and Drug Administration) that the investigation may be successful. MPEP 2107.02. It is respectfully submitted that acceptance for human clinical trial is not only evidence of the asserted therapeutic utility, but also some objective evidence of nonobviousness.

A statement listing the trials can be found at [13]
http://www.geneticimmunity.com/pages/906725/index.htm
A Declaration to this effect was offered by the Applicants, and the offer was ignored.

[13] Evidence Appendix 8A

Note: Examiner's Contention – Antigen Presenting Cells

Claim 23 has been amended so that the complex must target antigen presenting cells. This limitation must now be given patentable weight. The Examiner states without offering any rationale that "transfecting antigen presenting cells" "may not occur." The applicants have pointed out that the evidence that "transfecting antigen presenting cells" has occurred is in the text of this case and is copious. With the claims amended so that the limitation is in the body of Claim 23, this limitation must be considered. MPEP 707.07(I).

Examples in the application present evidence that APCs were indeed transfected. They were transfected *in vitro* using the best available prior art material for antigen presenting cells, lipofectamine (Example 1, plasmid DNA encoding HIV-1/LWint-, an integration and replication defective HIV described in one of the inventors' other applications, showing production of various proteins; Example 3 demonstrates that these cells developed the desired CTL response *in vitro*). They were transfected *in vitro* using PEI, showing that PEI worked better than lipofectamine, in Example 5. The PEI- transfected cells were shown to produce an *in vivo* CTL immune response in Example 4.

DC were transfected with a plasmid encoding green fluorescent protein and a variety of other adjuncts *in vitro*, including various PEI derivatives (Example 6).

Antigen presenting cells were transfected according to the method in Example 8, using a plasmid encoding green fluorescen protein where the claimed complexes were applied to the skin of mice (page 22, line 37) and then skin samples were tested for transduction of Langerhans cells, and it was found that a sugar modified gene delivery system is preferred to transduce antigen presenting cells. (page 23, lines 19-20). Example 9 shows that the claimed complexes also migrated to the lymph nodes and expressed protein (page 24, lines 6-7).

Note: Motivation

The Examiner's citation of the Holler reference for a desire to use attenuated HIV as a raw material for vaccines does not supply the teaching needed to derive the claimed method. Further, the cells that were transfected were cancer (lymphoblastoid) cells in vitro, by electroporation. It says nothing about transfection of antigen presenting cells *in vivo*, by applying a formulation on the skin. Given the disclosure in the present application, and in the references cited therein, that such methods of transfection were not effective, and that barriers to

gene transfer vary with cell type, this reference cannot be said to supply either the kind of specific teaching or the expectation of success required to support an obviousness rejection.

D. Conclusion

In view of the above analysis and the evidence, it is respectfully submitted that the present rejection is inapplicable to the amended claims because neither the individual references nor their combination yield the claimed invention, and the case is well-supported by experimental results and secondary considerations, the impact of which have been acknowledged by peer-reviewed publications and the United States Food and Drug Administration.

7. Request for Relief

The present application was filed with a disclosure meeting all the requirements for patentability of the claimed invention, including a description of the closest prior art, the differences between the prior art and the claimed invention, the advantages of the claimed invention over the prior art, and experimental evidence demonstrating the connection between the claim limitations and the advantages of the invention. Further, the application was filed with an example showing not just the claimed result (transfection of cells) but also a therapeutic response (CTL response) and a Declaration and peer-reviewed publications of record confirming various statements within the text of the specification. This divisional application was filed February 21, 2002, based on a parent application filed September 15, 1998 and the presently claimed invention is now in human clinical trials in two countries.

The United States Patent and Trademark Office ("USPTO") has made no secret that inventions in the present field of endeavor have been subjected to "enhanced scrutiny" but has been unable to articulate, upon repeated, written requests from this and other applicants, what actions on the part of an applicant, what amendments, or what demonstration of patentability can meet that standard. Now the USPTO is offering a fast-track examination process for applications that meet the same criteria as this application, but for the field of endeavor.

As a result of a policy decision by the USPTO, the present inventors have been subjected to extraordinary expense and prosecution timelines, and in addition, have suffered from a loss of patent term of several years' duration. This application should receive a term extension equivalent at least to the passage of time from the file date to the projected issue date of the present set of Claims.

Conclusion

For all the above reasons and amendments, it is believed that all the Examiner's legitimate concerns have been fairly met. Favorable consideration is solicited.

Respectfully Submitted,

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